Docket No.: 350292000800
Client Docket No.: E910-US
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Masahiko MIHARA

Application No.: 09/381,598

Filed: March 20, 1998

Art Unit: 1646

For: PREVENTATIVES OR REMEDIES FOR SENSITIZED T CELL-RELATED DISEASES CONTAINING IL-6 ANTAGONISTS AS THE ACTIVE INGREDIENT

Examiner: J. F. Murphy

DECLARATION OF MASAHIKO MIHARA PURSUANT TO 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Masahiko Mihara, declare as follows:

1. I am the sole inventor of the above-referenced patent application, and am familiar with the contents thereof.

2. I have conducted experiments demonstrating the efficacy of the anti-IL-6 receptor antibody in a murine model for multiple sclerosis. The murine model for multiple sclerosis is the Experimental Allergic Encephalomyelitis (EAE) model and is regarded by practitioners in the field regard as a relevant model for multiple sclerosis. *See, e.g.*, Suen *et al.*, J. Exp. Med. 186: 1233 (1997) (already of record). The experimental results are set forth in the paragraphs below.

EXHIBIT A

3. Induction of EAE: Mice of 8-10 weeks of age were subcutaneously injected in the abdomen with 300 μg of myelin oligodendrocyte glycoprotein ("MOG") peptide (MEV GWY RSP FSR VVH LYR NGK) in complete Freund's adjuvant ("CFA") (containing 500 μg *Mycobacterium tuberculosis*) (Becton Dickinson) as previously described. *See* Suen *et al.*, J. Exp. Med. 186: 1233 (1997) (already of record). Mice simultaneously received 500 μg pertussis toxin (ALEXIS' Biochemicals) intravenously via the tail vein. Two days from the immunization, mice received an additional 500 μg pertussis toxin via intravenous injection into the tail vein. Seven days later, mice received an additional subcutaneous injection of the MOG peptide (300 μg) on the opposing side of the abdomen of the mouse as the first immunization (three mice per one group). The mice were observed daily and scored on a scale of 0-5 with gradations of 0.5. No clinical signs received a score of 0; loss of tail tone received a score of 1; wobbly gait received a score of 2; hind limb paralysis received a score of 3; hind and fore limb paralysis received a score of 4; and death received a score of 5. Each mouse was scored, and an average for each group was calculated. The anti-IL-6 receptor antibody, MR16-1, was injected once intraperitoneally at the same time as the first immunization. A control group received an injection containing only PBS.

4. Results: The results of the study are shown in Figure 1. Control mice (receiving only PBS) developed clinical manifestations similar to those previously reported with a chronic and severe paralysis and weight loss. *See, e.g.*, Suen et al. The control mice demonstrated clinically significant symptoms as early as day 3 post injection with the majority of mice progressing to hindlimb paralysis by day 12. Clinical signs continued for the duration of the observation period (day 34) with only mild abatement of the paralysis, *i.e.*, clinical scores of 4-5. In contrast, a single administration of the anti-IL-6 receptor antibody prevented clinically significant symptoms until day 10 post-injection. Moreover, the antibody reduced the severity and the duration of the clinical symptoms experienced. For example, the antibody resulted in clinical scores between 1 and 2 after day 22. These results demonstrated that a single administration of an anti-IL-6 receptor antibody suppressed both the development and clinical severity of EAE.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Executed at Tokyo, Japan, on April 11, 2005.

Masahiko Mihara

Figure 1